A new dawn in social insect research: small solutions for big problems in biology

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Abstract

Social insects are long-standing models for understanding social behaviour and evolution. Recent methodological and theoretical innovations are driving a step change in the questions that social insects can be used to answer. Here we examine some of these current trends and attribute them to advances in molecular biology and tracking technology, a widening perspective of the model organism, and the emerging concept of the superorganism. We identify some of the key challenges, and outline nascent solutions as offered by leaders in the field. Whilst far from exhaustive, our review showcases examples of the potential offered by social insect research to current problems in biology, and encourages open-minded exploration of one of the richest and most complex of biological phenomena: sociality.

Introduction

The social insects - the ants, aculeate wasps, termites, and certain bees - have inspired biologists for over two thousand years [1]. We believe that the study of social insects is currently undergoing a conceptual and methodological renaissance. This new wave of social insect biology is extending into unconventional fields of biology, from immunology to plasticity. As a result, social insects now offer powerful testing-grounds for emerging trends in biology that are of deep interest to biologists across the research spectrum.

The history of life can be read as a series of 'major evolutionary transitions', in which individual units come together to form a single whole [2, 3]. These transitions are governed by similar principles, and include the origin of the genome from solitary strands of nucleic acid and the evolution of multicellularity from free-living cells. The evolution of insect sociality represents one of these major transitions, as independent individuals came together to form colonies. As seen in the origin of multicellularity, insect colonies have evolved complex colony-level traits. Unlike tight groups of cells, however, insect colonies are in many ways more accessible and readily open to fine-scale experimental manipulation.

The recognition that insect colonies echo the fundamental principles of the major transitions opens new vistas for social insect research. As a result, insect colonies are now being treated as models for questions as diverse as the evolution of innate immunity [4], the links between plasticity and adaptation [5], how specialist phenotypes can emerge from a common genome [6], the emergence of heritability [7], and the roles played by personality and heterogeneity in biological systems [8].

In this review, we aim to identify some key general areas of debate or challenges in biology, and outline how social insect research is helping to solve these by offering innovative, concept-changing solutions. Our approach to this task involved exploiting the expertise of 100+ social insect biologists at the 2015 meeting of the North West European Section of the International Union for the Study of Social Insects (IUSSI). Delegates were asked to nominate key general topics in biology that social insect research is – or is moving towards – centre-stage on. Our experts then came together in workshops to define the conceptual advances offered by social insect research, and discuss emerging challenges and solutions. Here, we focus on the four emerging trends that most strongly reflected the research activities of our 100+ experts - and which serve as just an appetiser of the range of key challenges in 21st century biology to which we believe social insect research will make a significant contribution.

Trend 1: Superorganisms and a unified theory of social evolution

The problem

The evolution of societies with sterile worker and reproductive queen castes is the latest major evolutionary transition. Inclusive fitness theory has explained the complex interplay of kinship, conflict and cooperation in the evolution of such societies. While this transition means that selection largely functions at the colony level, we understand surprisingly little how evolution of group level traits compares to evolution of individual traits that have been the traditional focus of evolutionary biology.

Conceptual advances

Comparing insect societies (as "**superorganisms**") to complex multicellular organisms is compelling, and dates to the early 1900s [9]. The major transitions view calls for taking this seriously. The superorganism view has often been limited to physical and functional analogies, but it can provide an explicit comparison of new kinds of organisms, guiding a more unified understanding of how evolution at different hierarchical levels works [3].

Challenges and solutions

To understand how traits of superorganisms evolve, and the similarities between evolution at different levels, we need to understand of the extent to which fitness effects and heritability of variation are colony-level traits, which constitute the building blocks of natural selection at the superorganism level. Colonies vary consistently in traits such as reproductive allocation [10, 11], foraging and nest site decisions [12]. Fitness effects of variation in aspects of colony phenotype such as caste ratios, genetic diversity, colony sizes and foraging thresholds have been demonstrated experimentally and in the wild [12], using fitness proxies such as foraging success or disease resistance. In order to set the colony-level variation into an evolutionary context, we need to understand how colony traits respond to selection.

While heritability and responses to selection of group traits are poorly understood [13], we outline three areas where significant progress can be made. Firstly, long-term field studies allow mapping fitness effects at the levels of life-time reproductive output and recruitment [14] and heritability of colony traits [7]. Colony longevity, long dispersal distances, and pervasive plasticity in colony traits, however, make such field data challenging. This is why, secondly, experimental approaches are needed. Experimental work in honeybees [15, 16] and ants [17] has shown the complex genetic architecture of traits such as foraging preferences and allocation decisions, and increased use of ant lab models promises to advance and broaden this area [18, 19]. Thirdly, theory needs to reveal how traits of superorganisms with internal genetic diversity, extensive phenotypic plasticity, conflicts of interest between individual and group levels, complex indirect genetic effects [20] and caste antagonistic selection pressures [21] respond to selection. To achieve this, a quantitative multi-level selection view has to merge with the optimality logic of inclusive fitness theory [22] instead of pitting the two against each other. Finally, how individual-level variation translates (during ontogeny, and in the genotype-phenotype map of the superorganism) into colony-level variation is non-trivial, and continues to be modelled extensively (see [23], and references below).

The concept of a superorganism combines disparate approaches to a general framework of studying how traits of colonies evolve. There is potential in comparative tests of adaptive hypotheses over key eco-evo traits [3] and analyses of ongoing selection of superorganismal traits, both in the field and in the lab. The variation in insect societies (Box 1), and the mechanistic understanding of the behaviour of the parts that make the whole, provides an excellent setting for taking the study of evolutionary transitions to the next level, to understand how a transition in heritable fitness-relevant variation happens [24] and to compare evolutionary processes across hierarchical levels.

Trend 2: Biological heterogeneity is functional, not just noise

The problem

Increasingly, studies of **heterogeneity** in biological systems – including at a cellular level within organisms – have found functional benefits [25, 26]. However, it is often unclear in which cases variation is actually adaptive, and in which cases it is simply 'noise'. As superorganisms at varying levels of sophistication, social insect colonies provide good models for investigating this in relation to behaviour, due to their (often) advanced division of labour, and propensity for self-organisation [27]. The questions that have traditionally been asked have been shaped by the dichotomy of task partitioning (i.e. different worker tasks and reproductive commitment). Diversity (and intra-caste specialisation) among workers promotes colony efficiency and productivity [28]. However, recent studies have revealed extraordinary heterogeneity *within* each specialist phenotype. To what extent is this heterogeneity adaptive for the superorganism, and how?

Conceptual advances

The heterogeneous nature of social phenotypes within the colony appears to be adaptive, with impacts on colony-level functioning. Consider temperature regulation in honeybees: genetically-diverse colonies sired by several males have less excessive colony-level responses to temperature fluctuations, because the response thresholds of individuals are more variable [28]. Such variation in sensitivity is also important in decision-making. For example, amongst house-hunting ants, some individuals have high thresholds for accepting a new nest – they continue looking for alternatives when many of their other nest-mates may be satisfied [29] making it more likely that the colony finds the best available option [30]. At the same time, lower threshold individuals give stability to the ants' location, as they are less easily persuaded about the need to move. Thus, even small differences in thresholds may be sufficient to drive important effects. Social insects lend themselves to answering these general questions at a fine-scale, due to their large colony sizes, observability, and some recent breakthroughs in individual-level automated monitoring (Box 3).

Challenges and solutions

We identify five aspects of biological heterogeneity that remain key challenges of general relevance to biology, and that social insects can offer insights into. First, some forms of heterogeneity are clearly important for colony functioning; but are *all* forms important? If not, why not? And if so, are they inter-related? Examples include corpulence, with several ant genera displaying very heterogeneous distributions of fat stores even in the brood [31, 32], and activity levels, with some workers doing very little work and others conducting a large proportion [33].

Second, how is heterogeneity linked across organisational scales? Social insects allow us to examine how colony-level variation may emerge from intra-colonial heterogeneity. Behavioural syndromes (consistently associated behavioural traits) are observable at the colony-level, but it is not clear how these relate to individual-level heterogeneity [34, 35]. Indeed, it has been suggested that heterogeneous traits at the colony-level may be most important for species-level ecology, allowing for the co-existence of complementary types [12].

Third, the distributions of heterogeneity are unlikely to be normal. This is a problem, since models of collective behaviour often assume that group members are homogeneous, even when the group size is large. In decision-making contexts, where non-uniform distributions of preferences occur, normal distributions are often assumed [36], but skewed distributions are more likely [37]. Measuring and manipulating the distribution of heterogeneous traits is possible using social insect workers, particularly ants [33]. However, in order to maximally exploit this, we require a better understanding of how flexible individual thresholds are in response to the removal of workers in social networks, and over what timescale [38].

Fourth, if heterogeneity is functional, it must be a target of selection, but the mechanisms by which this occurs are unknown. Social insects offer insights into the role of life-history and social information in generating and maintaining adaptive heterogeneity. Life-history factors include physical maturity, nutrition and environmental influences [39, 40], while social factors include learning and experience [41]. Exploitation of individual tracking technology opens avenues for future research, such as behavioural tracking over long periods of time under different mechanistic scenarios, to investigate how thresholds may be altered (Box 2). Use of social information also shapes individual-level variation in behaviour [42, 43], and may be modulated in certain social insects by their motor planning function [44]. However, whether a social insect colony can make use of the most relevant information from all members depends on the context, with speed-accuracy tradeoffs coming into play [45]. Furthermore, social information is not always reliable, due to conflicts between the group and individual [46].

Finally, **plasticity** is likely to be an important adaptive trait of heterogeneity. For example, theory predicts that evolution can favour mixed populations where most individuals rely heavily on social information and a few rely on individual information [47]. However, there is only limited evidence to suggest that animals can effectively decide whether to use social or personal information, when the two are in conflict [48]. Social insects are ideal candidate systems to investigate how evolution has resolved this trade-off, as they constitute easily manipulated units that make use of both information types. It is here that two of the above themes are linked, as there may be some degree of heterogeneity in individuals' social information use strategy, and indeed, ability [49, 50]. Furthermore, a recent developing concept in collective animal behaviour is that of 'keystone' individuals, whose importance to their group is comparable to the role of a keystone species in an ecological community. These are individuals who play a critical role in successful group behaviour, through dominance, leadership, or spreading information [51]. In relation to the question of biological heterogeneity in the social insects, the value of the keystone concept could also be considered. Future experiments involving manipulations of the distribution of individual strategy types will be invaluable (e.g. using molecular methods, Box 3), and will contribute to our understanding of the benefits provided by individual heterogeneity and plasticity.

Trend 3: Phenotypic plasticity is key to coping with challenging environments

The problem

Phenotypic plasticity is an adaptive mechanism whereby a single genome can respond to environmental challenges by expressing different phenotypes under different environmental conditions [52]. Such plasticity is especially important in rapidly changing environments where there is not time for populations to evolve or disperse. Plasticity is understood well at the behavioural and physiological levels, but the molecular mechanisms underlying plasticity are poorly explored. Studying these processes is essential for understanding how organisms respond to their environment, and predicting how they will cope with environmental change. Social insects represent a unique opportunity to engage in these studies: they display well known patterns of plasticity in their behaviour and physiology at the individual and group level, they are easily amenable to laboratory and genetic manipulation and there is increasing availability of genomic resources for several species (Box 3).

Conceptual advances

Social insect castes are an exemplar of phenotypic plasticity, a superorganismal trait closely analogous to the separation of germ cells and somatic cells in the organism: reproductive and non-reproductive individuals develop via differential expression of shared genes [53, 54]. Social insect species with simple societies are especially useful for studying phenotypic plasticity, as they retain caste flexibility throughout life, such that adults can respond to changes in their environment by

switching caste (Box 1) [55, 6]. Recent advances in molecular biology have opened new opportunities to understand caste differentiation at the level of the genes (Box 2), and are serving as enlightening models for revealing the mechanisms of phenotypic plasticity [56, 5, 53, 57]. For example, high levels of plasticity have been associated with subtle differences in transcriptional networks, molecular pathways, DNA methylation, and tissue-specific gene expression [6, 58]. In contrast, low levels of plasticity appear to be governed by large-scale shifts in molecular processes: functional enrichment and canalization of gene expression may limit plasticity [59].

Exploring the mechanisms of phenotypic plasticity in social insects is an opportunity to move the focus beyond a single individual, as plasticity is a feature that applies also to whole colonies. The network of interactions among colony members can reach different levels of plasticity, depending on fluctuating colony needs, similarly to what we observe in molecular networks of co-expressed genes. However, this aspect of social behaviour has not been fully characterized yet. Furthermore, from an applied perspective, understanding phenotypic plasticity in social insects has great value due to their role as both invasive species, and providers of ecosystem services which are threatened by environmental challenges [60]. The rich diversity of phenotypic plasticity in social insects (Box 1), together with new molecular tools (Box 2), place this taxonomic group centre stage in advancing our understanding of plasticity at multiple levels of complexity and the interplay with the environment.

Challenges and solutions

We identify three fundamental aspects of phenotypic plasticity that remain key challenges of general relevance to pure and applied biology, and that social insects offer insights into. Firstly, we understand little about the molecular processes underlying plasticity. The tractability of experimental work in the field and lab (Box 1) and their small, accessible genomes (Box 3) make social insects good models for this. The range of plasticity exhibited by social insects (Box 1), across different developmental stages offers much potential to pick apart the mechanisms of plasticity. This will generate the fundamental knowledge required to address urgent challenges to biodiversity in a rapidly changing environment.

A second timely challenge is the need to understand the causes of variation in plasticity. Climate change means that organisms are increasingly faced with sudden unexpected environmental challenges. Whilst some are able to adapt to novel environments (e.g. invasive ant species [61]), others are threatened by ongoing challenges such as habitat loss, and novel toxins or pathogens [62]. We hypothesise that: 1) variation in genomic plasticity may explain differences in the way social insects react to environmental change; 2) the level of plasticity may vary depending on the nature of the challenge (e.g. chemical, temperature fluctuation, pathogens). Using a comparative approach (Box 1) and exploiting increasingly accessible molecular tools (Box 3), traits that explain variation in plastic responses may be identified, and used to advise biodiversity management.

A third urgent challenge is to understand when phenotypes become more plastic. This is timely as invasive species (which display high levels of plasticity) threaten natural ecosystems globally. For example, is plasticity a pre-adaptation to invasion or does plasticity emerge due to the invasion process? Or, can molecular indicators of plasticity (e.g. epigenetic marks) predict vulnerability to environmental challenges and thus improve predictions (and mitigation) of population declines? Similar questions have been addressed in plants and occasionally in animals [63, 64, 65]. Using social insects to address these questions comes with added value: they are an ecologically and economically important group, as ecosystem service providers (e.g. pollinators, [66]), and in terms of the ecological risks they pose (as invasive species, e.g. [67]).

Understanding how social insects respond to environmental challenges promises to be invaluable for biodiversity management and protection of natural capital, as well helping address basic science questions on the nature of phenotypic plasticity.

Trend 4: Towards a unified framework for understanding disease defence

The problem

Research on the vertebrate adaptive immune system has led to major advances in our understanding of host disease defence [68]. However, despite the potency of immunological memory, it is often **innate immunity** that makes the difference between survival and death [69]. The discovery that invertebrates, including social insects, can prime their immune responses despite lacking adaptive immune machinery, and are able to transfer immune protection across generations, is paradigm-shifting [70, 71, 72]. Further, many animals including humans, live socially and there is an emerging view that social interactions can both negatively and positively affect disease dynamics [73, 74]. For example, although living in groups can be costly because susceptible and infectious animals will more frequently interact than in solitary species, cooperation and the evolution of collective behavioural defences can enhance disease resistance and tolerance in social animals [75]. In general, we lack a solid understanding of the interplay between physiological immunity, behavioural responses and social interactions, yet this is essential to the study of disease defence, host-pathogen coevolution and epidemiology [68].

Conceptual advances

Social insects offer an integrated approach for the study of disease defence. Unlike other animal societies, social insect colonies can be easily observed and manipulated in the laboratory and field, with high levels of replication. The natural variation and plasticity present in their colonies (Trends 2 & 3) makes them ideal candidates for eco-immunological work and powerful models to study the evolution of immune defences from the gene to the society [76]. With the diversity of social complexity in social insects (Box 1), we can study disease dynamics and epidemiology in societies of different sizes and social structures. Studying disease dynamics in social insects has already led to new concepts: “social immunity” is the additional layer of defence arising from collectively performed disease defences that reduce the disease susceptibility of the colony, rather than its individuals [77]. It is thereby analogous to the physiological immune system of multicellular organisms, and could provide insights into the evolution of immune defences across these domains [78].

Challenges and solutions

We identify four critical issues that are of general importance to the study of disease defence in animals. The first concerns the molecular mechanisms underpinning transgenerational immune priming without antibodies. The fact that mothers can enhance the resistance of their offspring against diseases without antibodies has been a puzzle and we are only beginning to understand the mechanisms behind it. For example, a mechanism for maternal immune priming in insects was recently discovered in the honeybee: egg yolk protein vitellogenin transfers bacterial fragments from food to developing eggs [79, 71]. However, we do not yet know what other mechanisms are at play. Do mothers also pass on other molecules (e.g. mRNA or specific proteins), which shape the phenotype of the developing embryo? It has also been argued that the evolution of immune priming in insects is dependent on the longevity of the species and mechanism of dispersal [80], but strong evidence is lacking. Social insects would be ideal candidates to test these hypotheses as they exhibit large variation in longevity and dispersal strategies.

Secondly, a better understanding of the costs of immune defence is required. There is a suggestion that resistance is traded off against other fitness traits and depends on life history [68]. Immune priming gives undeniable fitness benefits when faced with re-infection, but the costs remain unknown. In social insects with a strict colony structure and task division, we predict that the costs and need for immune defence would differ between colony members. With well-established model systems in social insects (Box 1) and a growing availability of genomic information (Box 3), we are able

to pinpoint the costs immune defences inflict on fitness-related traits, the interaction between physiological and behavioural immunity, as well as what kind of costs are linked to behavioural defences.

Thirdly, the adaptive significance and fitness effects of behavioural disease defences for the superorganism are untested or unknown in most cases. In order to study the impacts of behavioural defences on survival and fitness, longer-term studies are required and can be achieved using social insects with short generation times (e.g. [81]). Experimental manipulation of behaviour can be challenging, but is possible in the social insects. As an example, nest entrances can be closed to prevent the removal of corpses, a key social immunity behaviour [82]. Moreover, with the increasing number of sequenced genomes and RNAi-mediated knockdown techniques, genes that affect behavioural phenotypes can be targeted directly [83].

Finally, studies of complete social groups are needed to understand the role of social networks on disease transmission and susceptibility [84]. Social insects are ideal candidates for studies of epidemiological networks, given whole colonies can be observed and manipulated, and they are modulatory in the form of morphological castes and performance of tasks [85, 86, 87]. With the development of tracking techniques and advances in network analysis (Box 2), disease outbreaks can be studied across space and in real-time within the 'physiology' of the superorganism, providing fine-scaled monitoring of behaviours and changes in social interactions [84].

Concluding remarks

Social insect research is one of the historic triumphs of modern biology. A comprehensive body of natural history has been documented for a broad range of species. Theory explaining the mechanisms and evolutionary processes underpinning sociality is highly developed. Social insects have provided important models for studying behaviour, development, and cooperation for over a century. We believe that social insects now offer solutions to some of the new big questions in biology (Box 4). The recognition that sociality exists across the levels of biological organisation, a renewed fascination with the extraordinary diversity of the social insects, and a powerful new ability to exploit this diversity through technological and molecular advances (boxes 2 and 3, respectively) represent an exciting trilogy of recent trends, responsible for the rise of the social insects as small solutions to big problems in biology.

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Figure Legends

Fig. 1: The evolution of social insect 'superorganisms' echoes the evolution of organisms: both are major transitions in evolution. The recognition that these two processes represent parallel events in evolution opens new vistas in biology, as social insects can provide insights into research questions that were previously only the domain of cell biology, medicine, and molecular biology. (Rat hippocampus: Gerry Shaw, CC-licence, Wikimedia; army ants: Geoff Gallice, CC-licence, Wikimedia)

Fig. 2: Trends in research output per year, in biological, agricultural, and environmental sciences on *Scopus*.

Box 1: Diversifying model organisms: going beyond the honeybee (photos to be added)

Social insect diversity is astounding. Despite this immense variability, social insect research has historically focused on a relatively small coterie of accessible model organisms, in particular the honeybee (*Apis mellifera*). These traditional model organisms are now facing increasing competition.

Models of simple sociality

In the quest to understand the evolution of sociality, it is crucial to study model organisms that are at an early stage of social evolution. These so-called 'primitively-eusocial' insects – including the *Polistes* paper wasps and halictid 'sweat bees' – offer windows into the conditions favouring group living. Workers in these species are not sterile, and the distinction between queen and worker is purely behavioural. As a result, they offer valuable opportunities to explore foundational questions in behavioural biology, including the role of plasticity in adaptation [88], the emergence of distinct personalities from a common genome [55], and the relative importance of direct and indirect fitness in the formation of social groups [89].

Models of complex sociality

Species with complex societies offer insights into the extraordinary level of coordination required to maintain functioning colonies of thousands of individuals. Fire ants (*Solenopsis invicta*) provide such a model. As an invasive species with dramatic ecological effects [90], they have become a focus in a high-stakes struggle to understand the role of sociality in invasions, and a multiplicity of genomic tools have been developed [91]. Remarkably, genetic research in *S. invicta* has uncovered the existence of a 'social chromosome' determining social structure [92], providing a rare example of the elusive 'greenbeard' traits predicted by evolutionary theory [93]. Similar advances are being made in the attine leafcutter ants of South America, where comparative genomics are revealing in fine detail the evolution of complex mutualisms [94].

Models spanning levels of sociality

Lastly, clade-level analyses offer the opportunity to make comparisons across different levels of sociality – something that would be impossible by focusing only on the honeybee. The bumblebee genus (*Bombus* spp.) provides a fascinating case-study: the genus includes species representing a range of social complexity, and certain species (in the subgenus *Psithyrus*) are workerless social parasites of the social *Bombus* colonies, invading foreign colonies, overthrowing the queen and exploiting the host workforce [95]. A new interest in genetic differences between these radically-different close relatives is revealing the genetic details of selection on social traits (e.g. [96]).

Box 2: The rise of automated monitoring

In recent years, developments in tracking technology have opened the way to more detailed study of collective insect behaviour. Time lapse video can be used to track aggregate activity levels over time (Boi, Couzin, Buono, Franks, & Britton, 1999), while individual trajectories of 2-dimensional movements can also be recovered from video using various tracking software (Pinter-Wollman, et al., 2013), though without persistent tracking of individual identity. Since different social insects are typically indistinguishable by eye, various marking techniques can be employed to track each individual's contribution to cooperative behaviours. Paint marks can be applied by hand to insects to give each a unique appearance (O'Shea-Wheller, Wilson-Aggarwal, Edgley, Sendova-Franks, & Franks, 2016), while radio-frequency identification (RFID) chips have been attached to ants to automatically monitor visits to particular nest locations (Sumner, Lucas, Barker, & Isaac, 2007; Robinson, Smith, Sullivan, & Franks, 2009). Tracking small ants over long distances is a challenge for a fixed-camera system, and so a moveable gantry-based camera can also be effective for detailed studies of individual movement (Hunt, Baddeley, Worley, Sendova-Franks, & Franks, Ants determine their next move at rest: motor planning and causality in complex systems, 2016). Recent research has successfully employed paper barcode tags to study the colony behaviour of a large species of ant (Mersch, Crespi, & Keller, Tracking individuals shows spatial fidelity is a key regulator of ant social organization, 2013); attaching tags becomes easier on larger insects. For flying insects such as bumblebees, small transponders can be attached and individually tracked using harmonic radar; this technique has been used successfully in long-term studies of their behaviour (Woodgate, Makinson, Lim, Reynolds, & Chittka, 2016). If individual animals are sufficiently different in appearance, manual marking or tagging may be unnecessary, with algorithms developed that can identify a 'fingerprint' based even on subtle differences (Pérez-Escudero, Vicente-Page, Hinz, Arganda, & de Polavieja, 2014). Since marking animals may modify behaviour, non-invasive tracking is clearly preferable. Furthermore, in relation to the question of biological heterogeneity, with ever-increasing video recording quality, even colonies of numerous, small and apparently identical insects may be revealed as a collection of behaviourally heterogeneous individuals.

Box 3: 'Omic' technologies

Recent advances in whole-genome high-throughput sequencing now facilitate testing of evolutionary and mechanistic hypotheses using a wide range of taxa, generating unprecedented datasets. Social insects have taken centre stage due to their ready availability and small genome size. Genomic, transcriptomic, epigenetic and proteomic approaches showcase the potential of these analyses in social insects to answer wider biological questions. For example, comparative genomics analyses have revealed the expansion of olfactory receptors in ants [97]; the identification of caste-associated gene expression has probed the molecular differentiation between simple and and complex social species [98, 6, 99]; epigenetic studies explain behavioural plasticity across social evolution [100, 101] and proteomic, lipidomic and metabolomic approaches are on the horizon. Despite the promise, considerable challenges remain in order that replicable, future-proofed datasets are produced. Methodologies need to be standardised at sample preparation, sequencing, data analyses and archiving stages to ensure comparability across studies and species. Here we provide a checklist of key parameters and tools to improve data comparisons.

I. Sample preparation

Collection in the laboratory versus field. The collection of samples *in situ* reflects the closest natural condition of the species. The natural environment is highly variable, while semi-controlled environments may result in unwanted laboratory side-effects [102]. The method should be determined by the biological question. For example, understanding the impact of diet on social organization requires laboratory conditions in order to quantify and measure the food intakes of the colony. However, it is important that the limitations of laboratory studies are recognised, particularly for behavioural studies and for studies which seek to be environmentally-relevant. It is crucial that both laboratory and field studies are consistent in their sample collection (same day, or at least same time of the day, careful monitoring of weather conditions) to avoid the introduction of environmental noise.

Pooled samples versus individual samples. Most sequencing studies use pooled samples, which averages the variability among individuals as well as increasing the amount of starting material, often essential for single tissues of tiny insects. The disadvantage of pooling is that individual-level phenotypic variation is lost. Use of pooled or individuals should be determined by the biological question being asked, and the quantity of starting material available. Where pooling is necessary, experimental design should seek to reduce such noise by controlling for these factors.

Whole-body versus specific tissue analysis. Whole-body analysis is of limited use in genomic studies due to the variability across tissues. In social insects, the analysis of highly specialized tissues, such as ovaries, will accurately reflect their specificity but will not necessary allow the identification of upstream signalling pathways linked with individual phenotype in relation with sociality. When analysing behaviour, brain tissue (either whole brains or dissected segments) is most widely used to characterize individual phenotypes. A high proportion of genes in the genome are expressed in the brain, and it has the most diverse population of RNA compared to other tissues [103, 104]. However, the heterogeneity of brain cells has led to the further isolation of specific brain segments for genomic studies [105, 106, 107].

II. Sequencing

Library preparations. RNA extraction and library preparation influences the quality and concentration of cDNA. For

example, sequencing the same sample at different concentrations produces greater variation [108]. Testing different kits (e.g. RNA only or combined RNA-DNA) and library preparation protocols (number of PCR cycles and concentration of adapters) are key for library optimization, although differences in RNA-Sequqencing library sizes can be mediated by normalization [109]. Further, external controls such the use of RNA spike-in may be extremely valuable for large-scale comparisons across different laboratories [110].

Batch effect reduction. Barcoding and sequencing barcoded mixed samples in multiple lanes can reduce batch effect [111]. The ENCODE project reported that RNA-Seq from multiple tissues from human and mouse clustered by species and not by tissue, yet once the sequencing lane was taken into account, the reverse was found, highlighting the importance of normalizing for batch effect [112].

Sequencing Platform. Sequencing platforms have improved in consistency and reliability in recent years, but this has led to many differences in available data. The extent to which we can compare different platforms [113] suggests biases may be too great to adequately compare, e.g. between 454 and Illumina platforms. Therefore, it is important to choose a platform based on costs, reliability, but also on compatibility, comparability, repeatability, and future potential for exploiting archived data. With new platforms (or versions of) appearing almost annually, reproducibility across platforms is one of the major challenges facing the future of genomic studies.

III. Data analysis

Genome annotation and orthologs. Evolutionary analyses and cross-species gene expression analyses are annotation-dependent. Thus, the difference in annotation quality may hinder ability to detect genes gained and lost in evolution and biase gene expression across species. The use of single-copy orthologs (through KEGG orthology) to assess genome assembly and annotation quality might improve the analysis [114] as performed in *Solenopsis invicta* genome [101]. But, ultimately, the power (and relevance) of annotation is limited by the range of genes and species that have been subjected to functional genomic analyses.

Guanine-cytosine (GC) amino acid content. RNA-Seq is influenced by GC content and sequence length of genes (11). While these biases exist in analysis of differentially expressed genes (DEGs) within species, e.g. detecting DEGs between castes, the same biases also occur when comparing orthologous gene expression between species, because GC content and sequence length may differ between orthologs. Therefore, correcting gene length (for example, only comparing the gene expression level of aligned orthologous regions) and adjusting GC content difference between orthologs will be necessary before downstream analysis [115, 116].

In conclusion, the power of “omics” technologies, combined with the variability of social insect biology presents an invaluable tool for answering key biological questions. However, we require careful integration of needs and standards from ecologists, molecular biologists, and bioinformaticians in order to generate future-proof, comparable genomic data. accessible. The integration of fundamental ecological and evolutionary questions with molecular and computational advances provide an exciting step forward for sociogenomics – the study of sociality in molecular terms.

Box 4: Outstanding questions and challenges

Trend 1: Superorganisms and a unified theory of social evolution

Can the concept of the superorganism help reconcile the need for a unified theory of social evolution, that combines the logic of inclusive fitness theory with measures of response to selection at multiple levels?

How much variation occurs at a between-superorganismal level, and how does this influence life-history?

How do superorganism traits affect colony life-time fitness?

How heritable are superorganismal traits? How can colony-level traits be heritable with genetic heterogeneity, phenotypic plasticity and complex life-cycles?

Trend 2: Biological heterogeneity is adaptive, not just noise

Some forms of heterogeneity are clearly important for colony function; but are *all* forms important? If not, why not? If so, are they inter-related?

How is heterogeneity linked across different spatial scales?

What shape does heterogeneity take? Normality is assumed, but is unlikely to be realistic.

What are the mechanisms generating heterogeneity? What are the relative roles of genetics, life-history, social information use and learning?

How important are 'innovators' in the evolution of individual heterogeneity?

Trend 3: Phenotypic plasticity is key to managing environmental challenges

How is plasticity regulated at the molecular level?

What traits (genomic, life-history, environmental) explain variation in plastic responses to the environment?

What is the relationship between phenotypic plasticity and conservation, e.g. declining populations, resilience to environmental change and propensity to become an invasive species? To what extent can measures of plasticity be used to predict, and mitigate, changes in species ranges and abundance?

Trend 4: Towards a unified framework for understanding disease defence

1. What are the mechanisms behind trans-generational immune priming?
2. Which kind of costs are associated with immune priming?
3. What is the impact of behavioural disease defense on survival and fitness?
4. How do social network interactions influence disease transmission and susceptibility?

Glossary

Superorganism

A group of individual organisms that is sufficiently integrated that it can be seen as possessing the key characteristics of an organism itself

Phenotypic plasticity

The characteristic of developing different phenotypes in different environments (either adaptively or passively)

Transcriptomics

The study of messenger RNA molecules, which communicate the 'commands' of the genome to the cell for the creation of proteins

Major evolutionary transition

An event in the history of life in which lower-level units (such as solitary insects) come together to form a highly-integrated higher-level unit (such as a social insect colony). Other major transitions include the origin of the genome and the multicellular organism.

Biological heterogeneity

Differences between members of a group, which may be adaptive or passive

Innate immunity

The side of the immune system that presents general, non-specific responses to pathogens

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Figure 1

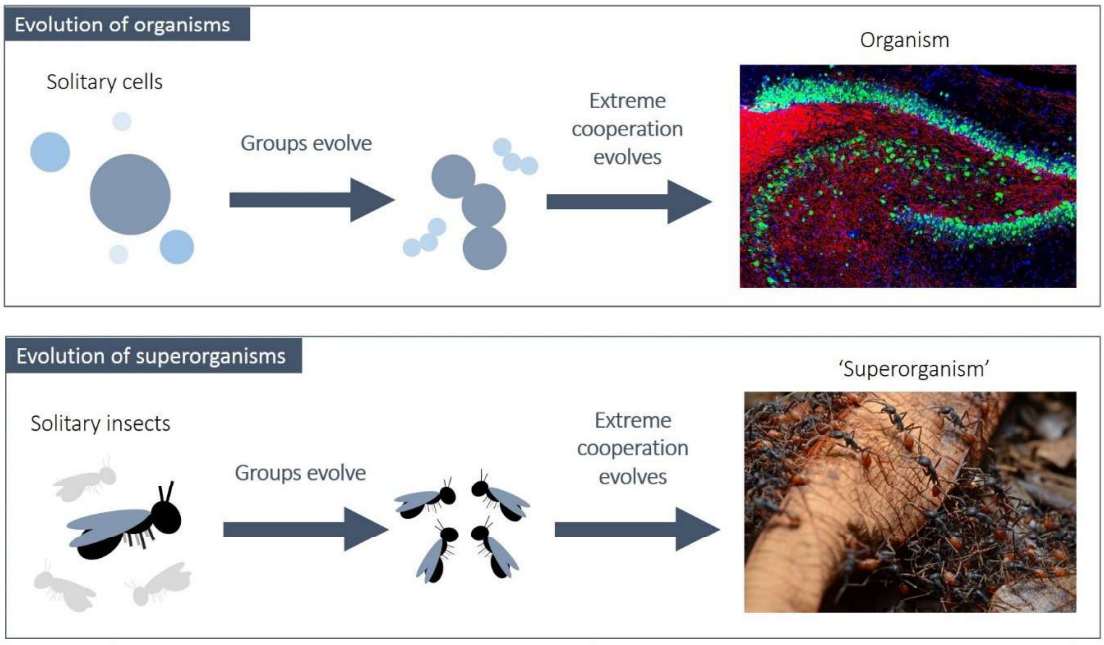
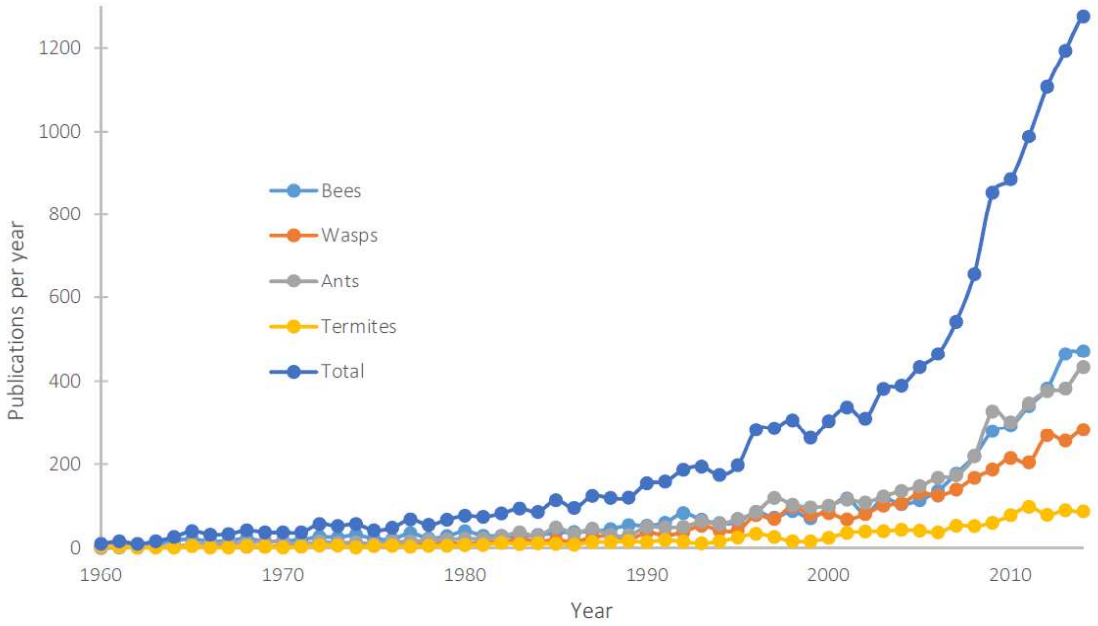


Figure 2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Publications per year
Bees
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Total
1960
1970
1980
1990
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2010
Year